Brief Report

GTP-Binding Proteins As Oncogenes in Human Tumors

by Frank McCormick*

Ras oncogenes are the most commonly known onco-genes in human tumors; their prevalence in different types of tumors and their role in the development of these tumors have been discussed in recent reviews (*1,2*). We have investigated the biochemical mechanism that accounts for the activation of *ras* proto-oncogenes to become oncogenes. In normal cells, the *ras* p21 cycles between the active guanosine triphosphate (GTP)-bound form and the inactive form, bound to guanosine diphosphate (GDP). The conversion of the GTP- to the GDP-bound form is catalyzed by a cellular protein re-ferred to as GAP (GTPase activating protein) (*3*). The mechanism by which the GTP-bound state is generated is not known; however, this mechanism must be much slower than the GAP-catalyzed reaction, so that most of the *ras* p21 in normal cells is in the GDP-bound state. In *ras*-transformed cells, the oncogenic mutant *ras* pro-tein exists in its GTP-bound state constitutively. This is because GAP fails to catalyze hydrolysis of GTP bound to these proteins. Oncogenic mutants can there-fore be thought of as mutants that escape from this aspect of GAP function.

Two types of GAP cDNA have been cloned from hu-man placenta (*4*). Figure 1 shows the structure of the proteins encoded by these cDNAs. Type I GAP is a 116,000 Da protein, with a hydrophobic amino terminus, two SH2 regions (*src*-homology region 2), one SH3 re-gion, and a catalytic domain comprising the C-terminal one-third of the protein (*5*). All cells express type I GAP, whereas type II, which lacks the first 180 amino acids of type I GAP and is generated by an alternative splicing mechanism, has only been detected in human placenta and in certain human cell lines, such as MRC-5 diploid fibroblasts.

Interaction of GAP with *ras* p21 may have conse-quences additional to conversion of the GTP-bound to the GDP-bound form. This possibility was raised by the

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discovery that GAP binds to a region of p21 that is necessary for *ras* function (*6,7*), suggesting that GAP itself might be part of a *ras* effector system. This hy-pothesis has not yet been proved, but additional support has been provided by the observation that oncogenic mutants of *ras* p21 retain their ability to bind GAP (*8*), even though binding does not lead to GTPase activation (i.e., downregulation). To date, no *ras* p21 mutant has been described that is biologically active yet fails to bind GAP.

Heterotrimeric G-proteins involved in signal trans-duction from hormone receptors to internal second mes-sengers cycle between GTP- and GDP-bound forms in a similar manner to *ras* p21 proteins. Again, the active form is the GTP-bound state. However, these proteins rely on intrinsic GTPase activity to convert active to inactive forms, rather than depending on an accessory protein such as GAP. Although the mechanism of down-regulation is therefore different, these proteins are also susceptible to constitutive activation by point mutations that lock them in the GTP-bound state. Landis and co-workers described point mutations in the G-protein al-pha subunit (the GTP-binding subunit) in human pitui-tary tumors (*9*). These mutations occurred at arginine-201 or glutamine-227. Arginine-201 has no exact hom-olog in the *ras* p21 protein, although it is in a region of Gs-alpha that is equivalent to the GAP-binding region. Glutamine-227 is the equivalent of glutamine-61 in *ras* p21 (a frequent site of oncogenic activation). Both types of mutation reduce intrinsic GTPase of Gs-alpha, as ex-pected for activating mutations.

To extend these observations, we have used poly-merase chain reaction (PCR) to amplify genomic DNA from over 300 tumor samples and have analyzed the frequency of mutations in Gs-alpha in codons 201 and 227 (*10*). Mutations at these positions occurred in pi-tuitary adenomas (43% of samples tested) and in thyroid tumors (4%). We have also initiated a survey of other G-protein alpha subunits that may be activated in hu-
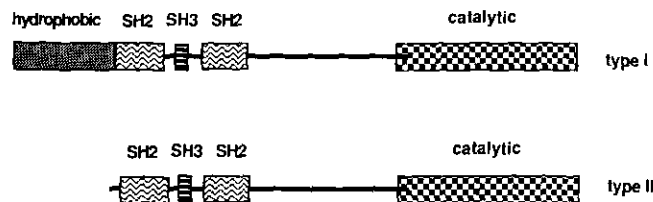
FIGURE 1. Structure of proteins encoded by GAP cDNAs.

man cancers. Initially, we identified codons of Gi-2 alpha subunit that are equivalent to codons 201 and 227 of Gs-alpha. We then reanalyzed the panel of 300 tumor samples for point mutations at one of these positions. Of all the tumors tested, tumors of the adrenal cortex (27%), ovarian granulosa (33%), and medullary thyroid (9%) were found to contain Gi-2 mutations, in all cases at codon 179, the Gi-2 equivalent of 201 in Gs-alpha. The substitutions produced by these mutations (arginine to histidine or cysteine) were the same as those of Gs-alpha codon 201, suggesting a common mechanism of activation.

For Gs-alpha, a major effector is known: this G-protein turns on adenylyl cyclase activity. Effector systems for Gi-2 are less clearly defined. Gi proteins were originally defined as G-proteins that inhibit adenylyl cyclase, but are now known to have other functions, which may be direct or indirect consequences of Gi activation.

In conclusion, striking parallels can be drawn between activation of *ras* genes in human tumors and activation of G-proteins. Both involve inhibition of GTPase activity, albeit by rather different mechanisms. As a result, the GTP-binding protein is locked in the GTP-state and signals constitutively. However, G-protein activation is so far confined to tumors of endocrine function, whereas *ras* mutations occur in most types of tumors. We presume that this means that *ras* p21 proteins activate pathways that are more intimately involved with cellular proliferation, whereas those of G-proteins are more specialized. In one case (Gs-alpha) the pathway is known, so that for the first time, an oncogene function can be understood in terms of the second messenger pathway it controls. We hope that pathways activated by *ras* proteins and Gi proteins will be elucidated in the near future.

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